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CONTRACTING ORGANIZATION: University of Nebraska Medical Center
Omaha, Nebraska 68198-6810

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DAMD 17-00-1-0361
Kenneth H. Cowan, M.D., Ph.D., Principal Investigator

I. The Breast Cancer Training Program at the University of Nebraska Medical Center

The Breast Cancer Training Program (BCTP) was established within the Eppley Cancer Research Institute of the University of Nebraska Medical Center (UNMC). As described in our application, the BCTP is a disease-specific component of our Cancer Research Training Program (CRTP). Since its inception in mid-year 2000, the BCTP has held monthly meetings and sponsored several breast cancer related seminars. Participation in BCTP activities is open to all interested students, fellows and faculty, as well as interested research and clinical staff. During year 1, we issued a request for applications from predoctoral and postdoctoral trainees for fellowship support from this training grant awarded by the Department of Defense Breast Cancer Research Program.

II. Breast Cancer Training Program Lecture Series

The purpose of the BCTP Lecture Series is to educate participating students, fellows and faculty on diverse topics directly relating to breast cancer. These lectures are held monthly (second Friday from 4:00 to 6:00 PM) and are attended by forty or more BCTP participants. BCTP faculty, fellows and students present these lectures. The schedule and program for this lecture series are summarized in Table 1.

Table 1. Schedule and Program for the 2000-2001 BCTP Lecture Series.

Date	Presenter	Topic
09-00-00	Dr. James Shull	Organizational Meeting of the BCTP
10-13-00	Dr. Kay-Uwe Wagner	Mammary Gland Development
11-10-00	Dr. James Shull	Rat Models of Mammary Cancer
12-08-00	Dr. Kay-Uwe Wagner	Mouse Models of Mammary Cancer
02-09-01	Mr. Tochacek, Ms. VanLith, Mr. Kohlgraf	Review of Research of Dr. Kate Horwitz
03-09-01	Drs. Dooley, Xie and Smith	Review of Research of Dr. Jeff Rosen
04-13-01	Dr. Kenneth Cowan	Breast Cancer Clinical Issues and Perspectives
05-11-01	Visiting Faculty	Cancer Biology Short Course: Tumor Immunology

III. Breast Cancer Seminars

The BCTP sponsored four seminars within the seminar series of the Eppley Cancer Institute during the 2000-2001 academic year. In addition, the BCTP hosted the Henry Lemon Memorial Lecture in Breast Cancer. These seminars are illustrated in Table 2.

Table 2. BCTP-Sponsored Breast Cancer Seminars.

Date	Speaker	Institution	Title of Presentation
10-19-00	Douglas Yee, M.D.	University of Minnesota	The IGF-I Axes and Breast Cancer
03-22-01	Kathryn Horwitz, Ph.D.	University of Colorado Health Science Center	Mechanisms of Hormonal Resistance in Breast Cancer
04-05-01	Jeffrey Rosen, Ph.D.	Baylor College of Medicine	Transgenic and Knockout Mouse Models of Breast Cancer
04-12-01	Joachim Liehr, Ph.D.	Stehlin Foundation	Genotoxic Mechanisms of Estrogen Carcinogenesis
05-24-01	V. Craig Jordan, D.Sc.	Northwestern University School of Medicine	Henry Lemon Memorial Lecture: Development of Antiestrogens for Breast Cancer Treatment and Prevention

IV. Cancer Biology Short Course

The BCTP, as part of the Cancer Research Training Program of the Eppley Cancer Institute, sponsored the annual Short Course in Cancer Biology, held May 7 through 11, 2001. The topic of the course was Tumor Immunology, and the course was taught by visiting faculty working in that area. The course consisted of a five-day series of lectures (2.5 hours each day) and chalk talks (2 hours each day). BCTP students and fellows are required to participate in this course, and students earn one semester credit hour for their participation. The visiting faculty and content for the course are illustrated in Table 3.

Table 3. 2001 Short Course in Cancer Biology.

Date	Speaker	Institution	Title of Presentation
05-07-01	Matthew Mescher, Ph.D	University of Minnesota	T Lymphocyte Response to Tumors
05-08-01	James Mule, Ph.D.	University of Michigan Medical Center	Tumor Vaccines
05-09-01	Jeffrey Weber, M.D., Ph.D.	University of Southern California, School of Medicine	Clinical Immunotherapy Trials
05-10-01	Ellen Vitetta, Ph.D.	University of Texas Southwestern Medical Center	Monoclonal Antibody Therapies for Cancer
05-11-01	Joyce Solheim, Ph.D.	Eppley Cancer Institute, UNMC	Course Summary and Evaluation

V. Support of Students and Fellows by the Breast Cancer Training Program

A Request for Applications (RFA) for stipend support from the BCTP grant was issued as soon as the award from the DOD Breast Cancer Training Program was finalized. Eight graduate students and five postdoctoral trainees applied for stipend support. An ad hoc committee was formed to evaluate the applicants. Three predoctoral and three postdoctoral trainees were selected to receive one-year stipends. The predoctoral and postdoctoral applicants are

Applicant	Award yes/no	Dept.	Mentor	Undergraduate Institution	Graduate Institution	UNMC
Alt, Jodi	No	CRTP, Path	Diehl, J.A.	B.A., Univ Nebr, Omaha, NE GPA = 3.59	M.A., Univ Nebr, Omaha GPA = 3.88	GPA = 3.00
Fulton, Jennifer	YES	CRTP, Path	Lewis, R.E.	B.A., Creighton University, Omaha, NE GPA = 3.58		GPA = 3.65
Harvell, Djuana	YES	CRTP, Path	Shull, J.D.	B.S., Clark-Atlanta University, Atlanta, GA	M.S., Clark-Atlanta University GPA = 3.381	GPA = 3.46
Kohlgraf, Karl	No	CRTP, Path	Hollingsworth, M.A.	B.A., Colorado College GPA = 3.57		GPA = 3.85
Shen, Xiaoling	No	CRTP, Path	Hollingsworth, M.A.	B.S., Beijing Normal University GPA = na		GPA = 3.00
Suranaryanan, Ganesh	No	CRTP, Path	Hollingsworth, M.A.	B.S., University of Bombay (India) GPA = na	M.S., University of Mumbai (India) GPA = na	GPA = 3.50
VanLith, Michelle	YES	CRTP, Path	Hollingsworth, M.A.	University of Mary, Bismarck, SD GPA = 4.00		GPA = 3.88
Wen, Yunfei	No	CRTP, Path	Hollingsworth, M.A.	B.S., Anhui Univ, Biology GPA = na	M.S., Univ Sci Technol of China GPA = na	GPA = 3.20

illustrated in Tables 4 and 5, respectively.

Table 4. Predoctoral Applicants for BCTP Stipends.

Abbreviations used: CRTP, Cancer Research Training Program; BMB, Department of Biochemistry and Molecular Biology; Path, Department of Pathology and Microbiology; UNO, University of Nebraska at Omaha; UNMC, University of Nebraska Medical Center. Grade point averages at UNMC are as of July 2000.

Table 5. Postdoctoral Applicants for BCTP Stipends.

Applicant	Award yes/no	Dept.	Mentor	Undergraduate Institution	Graduate Institution
Dooley, Constance	YES	Eppley Institute	Lewis, R.E.	B.A., Grinnell College, Grinnell, IA	Ph.D., University of Nebraska Medical Center
Gawron, Andrew	no	Eppley Institute	Hollingsworth, M.A	B.S., Truman State University, Kirksville, MO	M.S., Ph.D., University of Kansas, Lawrence KS
Kim, Jae-Huan	no	Eppley Institute	Rizzino, A.A.	B.S., Kon-Kuk University (S. Korea)	M.S., Kon-Kuk University Ph.D., University of Missouri
Smith, David	YES	Eppley Institute	Hollingsworth, M.A.	B.S., Microbiology Univ Newcastle upon Tyne	Ph.D., Univ. Newcastle upon Tyne, (UK) Postdoctoral Training, Univ. of Oxford, 1996-1999
Xie, Bin (Benjamin)	YES	Eppley Institute	Shull, J.D.	B.Med., M.Med Sci., Sun Yat-sen University of Medical Sciences	Ph.D., University of Hong Kong

VI. Reports of Research Progress by BCTP Predoctoral Fellows

A. Jennifer Fulton. Ms. Fulton has demonstrated that Kinase Suppressor of Ras (KSR) binds to the cytoskeletal component vimentin and continuously cycles through the nucleus. Data have been generated that suggest that nuclear localization of KRS enhances ERK activation.

Manuscripts:

Brennan, J.A., Volle, D., Chaika, O. and Lewis, R.E. Nucleo-cytoplasmic Transport Regulates the Biological Activity of *Kinase Suppressor of Ras* (KSR). In preparation.

B. Djuana Harvell. Ms. Harvell's research is focused on defining the role of estrogen in the development of pituitary tumors and mammary cancers and how these processes are modulated by dietary factors. She has demonstrated that the mammary epithelium of the genetically related ACI and COP rat strains exhibit quantitative and qualitative differences in their proliferative response to administered estrogen. More recently, Ms. Harvell has demonstrated that restriction of dietary energy consumption inhibits estrogen-induced mammary, but not pituitary tumorigenesis, in female ACI rats. Ultimately, the knowledge gained from these studies is expected to enhance our understanding of how dietary

energy consumption might influence the risk of cancers in humans for which estrogens are known to play a key role, such as breast cancer.

Manuscripts:

Harvell, D.M.E., Strecker, T.E., Tochacek, M. Xie, B., Pennington, K.L., McComb, R.D., Roy, S.K. and Shull, J.D. Rat Strain-Specific Actions of 17β-Estradiol in the Mammary Gland: Correlation Between Estrogen-Induced Lobuloalveolar Hyperplasia and Susceptibility to Estrogen-Induced Mammary Cancers. *Proc. Natl. Acad. Sci. USA*, 97(6): 2779-2784, 2000.

Harvell, D.M.E., Spady, T.J., Strecker, T.E., Lemus-Wilson, A., Pennington, K.L., Chen, F.S., Birt, D.F., McComb, R.D. and Shull, J.D. Dietary Energy Restriction Inhibits Estrogen-Induced Pituitary Tumorigenesis in a Rat Strain Specific Manner. In *Hormonal Carcinogenesis III* (eds. JJ Li, SA Li, JR Daling), Springer-Verlag, New York, pp. 496-501, 2000.

VanderWoude, E.A., Tochacek, M., Spady, T.J., Harvell, D.M.E., Snyder, M.C., Pennington, K.L., Reindl, T.M. and Shull, J.D. The ACI Rat as a Genetically Defined Animal Model for the Study of Estrogen-Induced Mammary Cancers. In *Hormonal Carcinogenesis III* (eds. JJ Li, SA Li, JR Daling), Springer-Verlag, New York, pp. 471-476, 2000

Harvell, D.M.E., Strecker, T.E., Xie, B., Pennington, K.L., McComb, R.D. and Shull, J.D. Dietary Energy Restriction Inhibits the Development of Estrogen-Induced Mammary Cancers, but Not Pituitary Tumors in the Female ACI Rat. Submitted.

Harvell, D.M.E., Strecker, T.E., Xie, B., Buckles, L.K., Tochacek, M., McComb, R.D. and Shull, J.D. Diet/Gene Interactions In Estrogen-Induced Mammary Carcinogenesis In The ACI Rat. J. Nutrition, 2001 (in press).

C. Michelle VanLith. Immune responses to MUC1, a tumor associated antigen found on epithelial tumors such as those of the breast, has been investigated as a target for tumor immunotherapy. The type of immune response generated against MUC1 varies depending on the tumor type expressing the antigen. CD4+ responses are generated against B16.MUC1, while CD8+ responses are generated against Panc02.MUC1. Vaccines utilizing interferon-MUC1 fusions are under development for use as a tumor vaccine. This vaccine will be tested in a MUC1 transgenic mouse model.

Manuscripts:

VanLith, M.L., Sivinski, C.L., Kohlgraf, K.G., Tempero, R.M., and Hollingsworth, M.A. MUC1-specific anti-tumor responses: molecular requirements for CD4-mediated responses. Submitted.

VII. Reports of Research Progress by BCTP Postdoctoral Fellows

A. David J. Smith. Dr. Smith has carried out studies investigating the regulation of the human *MUC1* gene. *MUC1* has been shown to be up-regulated in many forms of cancer including breast. He is also currently performing *in vivo* footprinting experiments to locate the positions of transcription factor binding sites in the promoter region of the *MUC1* gene. Finally, he is also developing a translational study in which cDNA array technologies will be used to compare gene expression profiles in primary breast cancers and associated axillary lymph node metastasis.

Manuscripts: none.

B. Bin (Benjamin) Xie. Dr. Xie has demonstrated that expression of progesterone receptor (PR) is much higher in the focal regions of atypical hyperplasia and mammary carcinoma induced in ACI rats by continuous treatment with estradiol than in normal or hyperplastic mammary glands. He has also demonstrated that expression of Cdkn2a is markedly down-regulated as an early event in estrogen-induced mammary carcinogenesis. Finally, Dr. Xie has established a series of immortalized mammary epithelial cell lines from the mammary tissues of ACI and COP rats and is determining whether or not these cell lines faithfully reflect the differing susceptibilities of the two rat strains to estrogen-induced mammary carcinogenesis.

Manuscripts:

Harvell, D.M.E., Strecker, T.E., Tochacek, M. Xie, B., Pennington, K.L., McComb, R.D., Roy, S.K. and Shull, J.D. Rat

Strain-Specific Actions of 17β-Estradiol in the Mammary Gland: Correlation Between Estrogen-Induced Lobuloalveolar Hyperplasia and Susceptibility to Estrogen-Induced Mammary Cancers. *Proc. Natl. Acad. Sci. USA*, 97(6): 2779-2784, 2000.

Harvell, D.M.E., Strecker, T.E., Xie, B., Pennington, K.L., McComb, R.D. and Shull, J.D. Dietary Energy Restriction Inhibits the Development of Estrogen-Induced Mammary Cancers, but Not Pituitary Tumors in the Female ACI Rat. Submitted.

Wong, Y.C. and Xie, B. The Role Of Androgens In Mammary Carcinogenesis. *Italian J Anatomy Embryology*, in press.

Xie, B. and Wong, Y.C. Sex Hormones In Mammary Carcinogenesis. *Acta Anatomica Sinica* 31:1-4, 2000.

Xie. B., Tsao, S.W. and Wong, Y.C. Sex Hormone-Induced Mammary Carcinogenesis In The Female Noble Rats: Expression Of Bax, Bcl-2, P53, And C-Myc In Mammary Carcinogenesis. *Breast Cancer Res Treat* 61:45-57, 2000.

Xie, B., Tsao, S.W. and Wong, Y.C. Isolation And Purification Of Breast Carcinoma Cell Lines From A Noble Rat. In Li JJ, Daling J, Li SA (eds) *Hormonal Carcinogenesis III*, Springer-Verlag, New York, pp.464-470, 2000.

Wong, Y.C., Xie, B., and Tsao, S.W. Induction Of Breast Cancer In Noble Rats With A Combination Of Estrogen And Testosterone. In Li JJ, Daling J, Li SA (eds) *Hormonal Carcinogenesis III*, Springer-Verlag, New York, pp.456-463, 2000.

C. Constance Dooley. Dr. Dooley is testing the hypothesize that ectopic kinase suppressor of ras (KSR) will inhibit the transformation properties of human cancer cells in vitro and the tumorigenic potential of mammary tissue in vivo. It is further hypothesized that the potency of KSR to inhibit tumorigenesis will be directly related to the ability of KSR to translocate to the nucleus. Dr. Dooley has successfully generated high-titer recombinant baculovirus for full-length KSR, KSR with two mutated phosphorylation sites, the carboxy terminal half of KSR, the amino terminal half of KSR, and two forms of KSR with reduced or absent activity. To correlate the subcellular distribution of KSR with a potential function with respect to transformation, MCF-7 cells will be infected with amphotropic retroviruses encoding KSR constructs. Infected cells will be evaluated by focus forming assays, colony formation in semi-solid media, and for the ability to form tumors in nude mice.

Manuscripts: none.

Key Research Accomplishments:

1. Harvell et al. demonstrated that dietary energy restriction markedly inhibits estrogen-induced mammary carcinogenesis in the female ACI rat.

2. Xie et al., demonstrated that down-regulation of Cdkn2a expression is an early event in estrogen-induced mammary carcinogenesis.

3. Van Lith et al., demonstrated that the type of cellular immune response directed toward human MUC1 differs as a function of the cancer type expressing the MUC1 transgene.

4. Fulton et al. demonstrated that the kinase suppressor of ras protein cycles in and out of the nucleus and interacts with the cytoskeletal protein, vimentin.

Reportable Outcomes:

Manuscripts submitted for publication:

Harvell, D.M.E., Strecker, T.E., Xie, B., Pennington, K.L., McComb, R.D. and Shull, J.D. Dietary Energy Restriction Inhibits the Development of Estrogen-Induced Mammary Cancers, but Not Pituitary Tumors in the Female ACI Rat. Submitted.

Manuscripts accepted for publication:

Harvell, D.M.E., Strecker, T.E., Xie, B., Buckles, L.K., Tochacek, M., McComb, R.D. and Shull, J.D. Diet/Gene Interactions In Estrogen-Induced Mammary Carcinogenesis In The ACI Rat. J. Nutrition, 2001 (in press).

Published abstracts from national and/or international meetings:

Harvell, D.M.E., Strecker, T.E., Xie, B., Pennington, K.L., McComb, R.D. and Shull, J.D. Dietary energy restriction inhibits estrogen-induced mammary carcinogenesis in the female ACI rat. Proceeding 10th Annual Research Conference of the American Institute for Cancer Research, Washington, D.C. August 9, 2000.

Smith, D.J. Analysis of DNaseI hypersensitive sites associated with the human MUC1 gene. 6th International Workshop on Carcinoma Associated Mucins, Robinson College, Cambridge, United Kingdom, 29th July- 2nd August 2000.

Van Lith, M.L. Characterization of MUC1-specific tumor immunity using animal models. Keystone Symposium on Cellular Immunity and Immunotherapy of Cancer. Santa Fe, NM, January 21-27, 2000.

Van Lith, M.L. MUC1-specfic anti-tumor response: distinct molecular requirements for CD4-mediated responses. Autumn Immunology Conference. Chicago, IL, November 19-21, 2000.

Van Lith, M.L. MUC1-specfic anti-tumor responses *in vivo*: distinct requirements for different types of tumors. Annual Meeting of the American Association of Immunologists. Orlando, FL. March 31-April 4, 2001

Xie, B., Buckles, L.K., Harvell, D.M.E. and Shull, J.D. Down-regulation of Cdkn2a expression is an early event in estrogen-induced mammary carcinogenesis in the ACI rat. Proceedings 92nd Annual Meeting of the American Association for Cancer Research, New Orleans, LA. March 24-28, 2001. Volume 42: 432, 2001

Other presentations:

DME Harvell, TE Strecker, M Tochacek, B Xie, KL Pennington, RD McComb, SK Roy, and JD Shull. Rat Strain-Specific Actions of 17β-Estradiol in the Mammary Gland: Correlation Between Estrogen-Induced Lobuloalveolar Hyperplasia and Susceptibility to Estrogen-Induced Mammary Cancers. *Clark Atlanta University Research Seminar Series, Atlanta, Georgia, January/2000.*

MUC1-specific anti-tumor responses: distinct molecular pathways required to reject different tumor types. Midwest Biomedical Student Research Forum. Feb. 19, 2000. Omaha, NE.